IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Charles T. Esmon and Naomi L. Esmon

SERIAL NO: 07/730,040 ART UNIT: 182

FILING DATE: July 12, 1991 EXAMINER: P. Hutzell

FOR: MONOCLONAL ANTIBODY AGAINST PROTEIN C

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

I, Armando D'Angelo hereby declare that:

1. I am Head, Coagulation Service at Hospital San Raffaele, Istituto di Ricovero e Cura a carattere Scientifico, Milano, Italy. I received my Doctor of Medicine degree from the Universita' degli Studi di Milano in 1977. I have conducted research in the area of hematology and blood coagulation since 1975.

2. Since my return to Italy from Dr. Esmon's laboratory in 1985, my group has attempted to reproduce a monoclonal antibody with the properties of "HPC4". The specific property we would most like to reproduce is the ion dependence of the binding of protein C, not involving the protein's Gla-domain, in view of its potential in the development of an assay system to measure protein C in plasma samples as indicated in D'Angelo, et al., J. Clin. Invest., 77:416-425, 1986 and Esmon, et al., Develop. biol. Standard, 67:75-82, 1987 (Attachment A).

3. Immunization of mice with human protein C according to the protocol described in Esmon, et al., Develop. biol. Standard, 67:75-

82, 1987 (Attachment A) was performed in collaboration with three different Italian laboratories with acknowledged expertise in the preparation of monoclonal antibodies.

4. These laboratories are: (a) Istituto di Chimica, Facolta' di Medicina, Universita'di Brescia (Head: Prof. A.Albertini, M.D.); (b) Sezione di Immunochimica ed Ibridomi, I.R.C.C.S. H. San Raffaele, Milano (Head: Dr. S. Marcovina, Ph.D.), and (c) Dipartimento di Biologia Genetica, Universita' degli Studi di Milano (Head: Prof. A. Siccardi, M.D.).

5. For the identification of clones with metal ion dependence of binding antigen, supernatants were screened by ELISA as described in D'Angelo et al., in (K. Okuda, ed.) "Automation and New Technology in the Clinical Laboratory", Blackwell Scientific Publications, 1990, pp 195-201, in addition to alternative protocols to avoid artifacts.

6. In spite of obtaining twenty three positive clones and the production of eight distinct monoclonal antibodies to protein C, none were dependent on the presence of calcium ions to bind protein C.

7. Our failure to reproduce a monoclonal antibody with the properties of HPC4, coupled with the absence of reports describing similar monoclonal antibodies in the literature, has led us to consider an alternative cumbersome and time consuming approach to develop this reagent in our laboratory in collaboration with Prof. Siccardi. However, we do not know if this alternative method will be successful.

8. It is my opinion that it would be very difficult, if not

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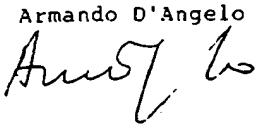
impossible, to isolate the same hybridoma antibody as HPC4, even after reviewing the Stearns, et al., J. Biol. Chem., 263(2):826-832 (January 15, 1988), Taylor et al., J. Clin. Invest., 79:918-925, 1987, and Esmon , et al., Develop. biol. Standard, 67:75-82, 1987 publications.

9. I declare that all statements made herein of my own knowledge are true. These statements are made with the knowledge that willful false statements are punishable by fine or imprisonment under applicable laws, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Armando D'Angelo



January 17th, 1992

Dr. Armando D'Angelo
Head, Coagulation Service,
IRCCS H.S.Raffaele,
Via Olgettina 60
20132 Milano, Italy